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(57) Abstract

Antibodies and antibody fragments which are anti–idiotypic to an antibody that interferes with the binding of the Cε3 region of IgE to its high affinity receptor (mimobodies), particularly, which are anti–idiotypic to BSW17 (BSW17–mimobodies) are described, as well as their use as pharmaceuticals, especially vaccines, in the treatment of IgE–mediated diseases.

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ANTI-IDIOTYPIC ANTIBODIES AGAINST ANTIBODIES WHICH INHIBIT THE BINDING OF IMMUNOGLOBULINE TO ITS HIGH AFFINITY RECEPTOR

The present invention relates to anti-idiotypic antibodies. It is directed towards the inhibition of interactions which would normally cause the triggering of mast cells and basophils induced by cell-bound immunoglobulin E (IgE) linked to an allergen, resulting in the release of histamine and other mediators as well as the de novo synthesis of cytokines involved in the regulation of allergic and inflammatory reactions. It concerns anti-idiotypic antibodies or antibody fragments that interfere with the binding of the Cε3 region of IgE to the high affinity receptor for IgE.

Knowledge of specific binding sites on IgE which interact with its high affinity receptor (FcεRI) provides a basis for the generation of antibodies which prevent this interaction by recognizing the binding epitopes. Induction of such antibodies by vaccination results in a novel and generally applicable therapy for allergy. The present invention describes the identification and production of, in particular, recombinant antibody fragments which can be formulated into a vaccine for the generation of anti-IgE antibodies which protect from induction of IgE-mediated allergic reactions.

Allergic symptoms are induced by the release of vasoactive amines (mediators), notably histamine, from cells into the surrounding tissue and vascular structures. Histamine is normally stored in special cells known as mast cells and basophil granulocytes. The mast cells are dispersed throughout animal tissue whereas the basophils circulate within the vascular system. These cells synthesize and store histamine within the cell unless a specialized sequence of events occurs to trigger its release.

The role of IgE antibodies in mediating allergic reactions is well known. IgE is a complex arrangement of polypeptide chains which, as in other immunoglobulins consists of two light and two heavy chains linked together by disulphide bonds in a "Y" shaped configuration. Each light chain has two domains, one variable (V_L) domain linked to a domain with a relatively invariant amino acid sequence termed a constant domain (C_L). Heavy chains, by contrast have one variable domain (V_H) and in the case of IgE, four constant domains (C_H1, C_H2, C_H3, C_H4, also known as Cε1, Cε2, Cε3, Cε4). The two "arms" of the antibody are

responsible for antigen binding, having regions where the polypeptide structure varies, and are termed Fab' fragments or $F(ab')_2$ which represents two Fab' arms linked together by disulphide bonds. The "tail" or central axis of the antibody contains a fixed or constant sequence of peptides and is termed the Fc fragment. The Fc fragment contains interactive sites which enable the antibody to communicate with other immune system molecules or cells by binding to their Fc receptors. Fc receptors are molecules which bind specifically to active molecular sites within immunoglobulin Fc regions. Fc receptors may exist as integral membrane proteins within a cell's outer plasma membrane or may exist as free "soluble" molecules which freely circulate in blood plasma or other body fluids. In the human system, high affinity binding of IgE to FcεRI is accomplished by a complex protein - protein interaction involving various parts of the third heavy chain constant region domain (Cε3) of IgE and the membrane - proximal immunoglobulin - like domain (α2) of the FcεRIα subunit. Although residues within the Cε3 domain of Fcε and regions belonging to the α2 domain of FcεRIα have been identified which are important for binding, the detailed mechanism of the binding process still remains to be characterized. Experimental evidence has shown that human IgE adopts a bent structure which is speculated to contribute to the high affinity of IgE for FcεRI ($Kd \cong 10^{-10}$ M). Moreover, this bent structure is also postulated to be responsible for the equimolar complex between IgE and cell bound or soluble FcεRIα, although the IgE molecule would provide identical epitopes on the two Cε3 domains for receptor binding. This monovalency is a functional necessity if receptor triggering in the absence of allergen is to be avoided.

Interactive sites, depending on their function, may already be exposed and therefore able to bind to cellular receptors. Alternatively, they may be hidden until the antibody binds to the antigen, whereupon the antibody may change in structure and subsequently expose other active sites which can then trigger a specific immune activity. A conformational rearrangement affecting Cε3 upon receptor binding has been proposed as an explanation for the 1:1 stoichiometry of the Fcε/FcεRI complex on the cellular surface.

The allergic (immunologic) release of mediators within the organism from the mast cells and basophils can only occur under the following circumstances: an IgE molecule must lock onto or attach itself with its Fc portion to the cellular Fc receptor site, thus securing the IgE molecule to the mast cell or basophil; and the Fab' portions of the cell-bound IgE molecules must be cross-linked by a particular compatible antigen (the allergen). Should such an interaction occur, the mast cell or basophil is automatically triggered to release histamine to

the local environment, manifesting familiar allergic symptoms (**Figure 1**). Other biochemical events follow in a late phase reaction, resulting in de novo synthesis and release of cytokines and other mediators.

Conventional approaches to allergy treatment have involved systemic therapy with anti-histamines or attempts to desensitize patients, approaches which have not adressed themselves to the basic IgE-mast cell/basophil interaction. Another approach has concerned itself with the production of polypeptide chains capable of blocking the binding of the IgE antibody to the Fc receptors on the cell surfaces and displacing IgE from binding sites upon which IgE is already bound, and investigated the nature of a putative "effector" site within the IgE Fc region which was speculated to provide an immunological signal which triggers mast cells/basophils for histamine release.

Using recombinant IgE fragments as immunogens for the generation of a protective anti-IgE vaccine has also been tried and shown to be effective. The main objection against such a vaccine results from the possibility that using large IgE fragments for immunization could initiate not only the production of inhibitory antibodies but also generate crosslinking and thereby anaphylactogenic antibodies in the patients.

A strategy to overcome this problem would aim at the identification of the smallest IgE fragment possible, ideally consisting of the receptor binding site only, which is buried within the IgE/IgERI complex after binding and therefore no longer accessible for crosslinking by the vaccine-generated immune response. Although attempts are still made this strategy is unlikely to be successful in view of the spatial distances of the various Cε3 regions involved in IgE/IgERI interaction.

The problems intrinsically linked to the "classical" vaccine approach may be overcome by using short mimotope peptides for active immunization, either as chemically synthesized peptides coupled to appropriate carriers, or as recombinant fusion constructs with e.g. ovalbumin or IgG. Such peptides are structural mimics of the epitope recognized by monoclonal antibody BSW17, which recognizes a conformational epitope on Fcε with a part of it residing within Cε3 and a part of it residing within Cε4. The hybridoma cell line producing BSW17 has been deposited on December 19, 1996 with ECACC under the provisions of the Budapest Treaty on the deposit of microorganisms, under **deposit number 96121916**. This antibody displays an interesting profile of biological activities as summarized in **Figure 2**. It is

by itself non-anaphylactogenic, and protects human mast cells and basophils from IgE-dependent histamine release induced by triggering agents. BSW17 or BSW17-like antibodies circulating within the vascular system protect from allergic reactions by a) inhibiting the triggering of mast cells and basophils through competitive inhibition of the IgE/IgERI interaction and b) by lowering serum IgE levels through downregulation of the IgE synthesis on the B cell level. As structural mimics of an anti-IgE antibody epitope, chemically synthesized BSW17 mimotope peptides induce an immune response which results in the production of BSW17-like antibodies in the host. Since BSW17 has been shown to be non anaphylactogenic, inhibitory to IgE/IgERI binding and IgE synthesis on B cells, these antibodies raised against the BSW17 mimotope peptide - based vaccine have similar protective properties. A possible disadvantage of such a BSW17 mimotope peptide - based vaccine may arise from the necessity of coupling the chemically synthesized peptides to carrier proteins to increase immunogenicity of the peptides. Furthermore, the structural flexibility of short peptides allows them to adopt many different steric conformations. Thus, only a fraction of the polyclonal anti-mimotope immune response will be therapeutically active by crossreacting with human IgE.

The present invention avoids the possible disadvantages intrinsic to a mimotope peptide approach. It is based upon antibodies or antibody fragments which are anti-idiotypic to antibodies that interfere with IgE-binding to its high affinity receptor, in particular upon a recombinant anti-idiotypic antibody to BSW17. According to Jerne's network theory (Jerne N., Ann. Immunol. 125C [1974] 373), hypervariable regions of an antibody (Ab1) can themselves act as antigens. The antibodies produced in this way are known as anti-idiotype (anti-id) antibodies (Ab2) since they bind to the idiotypic region of the first antibody (**Figure 3**). Such anti-id antibodies are directed to the binding site (paratope) of the first antibody (Ab1) and thus represent an "internal image" of the original antigen. Consequently, the anti-id antibodies (Ab2), termed internal image antibodies or Ab2β, are also capable of eliciting antibody formation via their hypervariable regions. These anti-anti-id antibodies (Ab3) structurally mimic the paratope of Ab1 and therefore have similar biological properties as the Ab1 antibody. In case of the hIgE/BSW17 system, IgE represents the original antigen and BSW17 the antibody Ab1. The paratope of an anti-BSW17 idiotype antibody Ab2 therefore represents a structural mimic of the hIgE region (the epitope) recognized by BSW17.

Structurally, the Ab2 paratope is equivalent to the chemically synthesized BSW17 mimotope peptides mentioned above. If such a (recombinant) anti-BSW17 idiotype antibody is used as a vaccine, a BSW17-like immune response (Ab3) will be induced in the vaccinated patient. Like BSW17, these (polyclonal) Ab3 immunoglobulins will interfere with the binding between IgE and its high affinity receptor, thus acting as anti-allergic agents. In contrast to flexible synthetic mimotope peptides, the Ab2 paratope will be presented to the environment in a structurally defined conformation. The immune reaction directed against the defined hIgE epitope will therefore be more specific. Moreover, no heterologous immunogenic carrier protein will be necessary. Possible side effects caused by a protein carrier like tetanus toxoid or diphteria toxoid, will therefore be avoided.

The present invention comprises antibodies or antibody fragments which are anti-idiotypic to antibodies such as E25 (olizumab) or CGP56901 or preferably, BSW17 that interfere with the binding of the Cε3 region of IgE to the high affinity receptor for IgE; they are hereinafter briefly named "**the mimobodies of the invention**". When they are anti-idiotypic to BSW17 they are hereinafter briefly named "**BSW17-mimobodies**".

The mimobodies of the invention are thus anti-idiotype antibodies or antibody fragments which specifically bind to an epitope that is the paratope of an anti-IgE antibody which recognizes the site on the Cε3 region of the IgE molecule that binds to the high affinity receptor for IgE (FcεRI).

The mimobodies of the invention are in principle of human origin, insofar as use in humans is contemplated. They preferably are recombinant. They preferably are monoclonal. They preferably are antibody fragments, e.g. consisting of or comprising:

- either both heavy and light chains (e.g. Fab fragments), or single heavy or light chains (e.g. light chain dimers), preferably together with their constant region component stretches, e.g. as defined in **Figure 4** (Seq.id. no. 35, 36, 37 and 38), whereby "constant region" is to be understood as also covering minor steric modifications, such as found in allotypic variants, e.g. at 1 to 5, normally just one, amino acid position in the constant part;
- or parts thereof, in particular at least the specificity-determining parts thereof, e.g. as defined in **Figures 5a** to **5d** (Seq.id. no. 2, 4, 6, 8);

- or subparts thereof, in particular at least the hypervariable subparts thereof, such as peptides made up of stretches of amino acids comprising at least one CDR, e.g. comprising at least one CDR, or preferably two, or more preferably the three CDR of **Figure 5a**, **5b**, **5c** or **5d** (Seq.id. no. 2, 4, 6, 8), optionally together with adjacent framework sequences, e.g. of up to about 10 amino acids at one or both CDR ends.

The mimobodies of the invention represent isolated and substantially pure antibodies or antibody fragments derived from naturally-occurring anti-id anti-IgE antibodies. In particular, they are substantially free of other antibodies. Under "substantially pure " is to be understood a purity of at least about 60 % by weight, preferably about 90 % by weight, more preferably about 99 % by weight or more.

The invention also concerns pharmaceutical compositions, especially vaccines, comprising mimobodies of the invention, either as a single molecular entity or as a protein conjugate chemically coupled to an immunogenic carrier molecule, where appropriate together with an adjuvant and further conventional excipients.

It further concerns the mimobodies of the invention for use as a pharmaceutical, in particular as a vaccine, in particular in the treatment of IgE-mediated diseases.

It further concerns the use of antibodies that interfere with the binding of the Cε3 region of IgE to the high affinity receptor for IgE, such as BSW17, for the identification of mimobodies of the invention, using conventional methods, such as phage display technology.

It further concerns a method of treatment of IgE-mediated diseases by, in particular, vaccination, comprising administration of a therapeutically effective amount of the mimobodies of the invention to a patient in need of such treatment or vaccination.

It further concerns the use of the mimobodies of the invention in the preparation of a medicament against IgE-mediated diseases, in particular of a vaccine.

It further concerns the use of the mimobodies of the invention for raising polyclonal or monoclonal antibodies thereagainst for passive immunization; the preparation of polyclonal or monoclonal antibodies against mimobodies of the invention for passive immunization, either by administration of mimobodies of the invention to a suitable non-human animal and isolation and purification of the antibodies generated thereagainst, or by conventional hybridoma technology; and the use of polyclonal or monoclonal antibodies whenever obtained from mimobodies of the invention in the treatment of IgE-mediated diseases by passive immunization.

It further concerns a process for the identification of the mimobodies of the invention which comprises

- identifying naturally occurring anti-idiotypic anti-IgE antibodies;
- isolating fragments thereof; and
- selecting recombinant fragments thereof by binding to a suitable anti-IgE monoclonal antibody, such as BSW17, which interferes with the binding of the Cε3 region of IgE to the high affinity receptor for IgE.

Once identified and characterized, the mimobodies of the invention may be prepared in conventional manner, e.g. by recombinant DNA technology or chemical synthesis.

For the identification of anti-idiotypic antibodies displaying the same specificity as the mimotope peptides mentioned above (i.e. the selected epitope on IgE), a bacteriophage display library can be used which is expressing the Fab part of a human antibody repertoire. This library is constructed e.g. from a pool of B cells obtained from tonsils of human subjects, and immobilized BSW17 antibody used as target for biopanning. Human Fab-expressing phage particles are isolated and enriched that specifically recognize BSW17. Thus these recombinant Fab fragments are mimobodies of the invention and represent anti-idiotypes against the hypervariable regions of BSW17. When used as a vaccine, they induce an immune response which results in the production of BSW17-like antibodies in the allergic patient. Since BSW17 is non-anaphylactogenic and inhibitory to IgE/IgERI binding and IgE synthesis on B cells, the polyclonal antibodies raised in the patient against the BSW17 anti-idiotypic Fab vaccine have similar properties. The immune response is very specific and safe since, in contrast to the "classical vaccine approach", no IgE-derived protein fragments are present which could generate crosslinking antibodies in the immunized patients, and compositions can be contemplated which are devoid of carrier.

These BSW17-mimobodies are recombinant antibodies or antibody fragments consisting of variable domains (V-domains) and constant domains (C-domains) derived from human immunoglobulin G. Two different clones (**clones 52** and **43**), which display different mimobody fragments on their surface, have been identified by biopanning of antibody phage libraries on immobilized BSW17 antibody. The mimobody structure which mimics the BSW17 epitope on human IgE resides within the hypervariable regions (CDR) and adjacent framework regions (FR) of the V-domains. The cDNA and amino acid sequences of the heavy and light chain V-domains of clone 52 and clone 43 are shown in **Figures 5a** to **5d** (Seq.id.no. 1 to 8).

The clone 52 light chain construct $(L.C.)_2$ consists of a dimeric "Fab-like" light chain fragment. The full structure of these BSW17-mimobodies is represented schematically in **Figure 4A** to **4C**, whereby the constant region parts thereof should be understood as also covering minor steric modifications such as found in allotypic variants as mentioned above. The amino acid sequence for each complete heavy and light chain of these clones is provided in **Figures 12a** to **12d** (Seq.id. no. 35 to 38).

The mimobodies of the invention possess pharmacological activity. They are therefore indicated for use as pharmaceuticals, e.g. as antigens for vaccines. While being substantially incapable of mediating non-cytolytic histamine release, they are capable of eliciting antibodies with strong serological cross-reactivity with the target amino acid sequences of the Fc region of IgE.

The initial dose of mimobody of the invention is e.g. from about 0.05 mg to about 5 mg, preferably about 1 mg; it will be administered e.g. nasally, or subcutaneously or intra-muscularly, followed by repeat (booster) doses of the same, e.g. 14 to 28 days later. Dosages to be used will depend to some extent on the age, weight and general health of the patient and may be adjusted as appropriate.

Direct vaccination, namely active immunization with the mimobodies of the invention will preferably be carried out using recombinant peptides (Fab fragment, light chain or heavy chain) which can be produced in various host expression systems, e.g. bacteria, fungi, or eukaryotic cells in conventional manner.

The administration of free recombinant mimobody is preferred. However, it is also possible to increase the immunogenicity of the immunogen further by chemical coupling to a immunogenic carrier. The term "immunogenic carrier material" herein includes those materials which have the property of independently eliciting an immunogenic response in a host animal and which can be covalently coupled to polypeptide either directly via formation of peptide or ester bonds between free carboxyl, amino or hydroxyl groups in the polypeptide and corresponding groups on the immunogenic carrier material or alternatively by bonding through a conventional bifunctional linking group. Examples of such carriers include albumins of animal sera, globulins of animal sera, thyroglobulins of animals, hemoglobins of animals, hemocyanins of animals (particularly Keyhole Limpet Hemocyanin [KLH]), proteins extracted from ascaris, e.g. ascaris extracts such as those described in J. Immun. 111 [1973] 260-268,

J. Immun. 122 [1979] 302-308, J. Immun. 98 [1967] 893-900 and Am. J. Physiol. 199 [1960] 575-578, or purified products thereof; polylysine, polyglutamic acid, lysine-glutamic acid copolymers, copolymers containing lysine or ornithine, etc. Recently, vaccines have been produced using diphteria toxoid such as CRM197 or tetanus toxoid as immunogenic carrier materials (Lepow. M. L., et al., J. Infectious Diseases 150 [1984] 402-406; and Coen Beuvery, E. et al., Infection and Immunity 40 [1983] 39-45) and these toxoid materials can also be used herein. In contrast to chemically detoxified diphteria toxin, the recombinant mutated diphteria toxin CRM197 is preferably used. In CRM197 the glycine-52 residue is replaced by glutamic acid, resulting in a non toxic product. CRM197 is a well characterized non-toxic carrier protein and is used in a registered human vaccine. The purified protein derivative of tuberculin (PPD) may also be used in the "active" immunization scheme since: (1) it does not induce a T-cell response itself (i.e. it is in effect a "T-cell hapten"), and yet it behaves as a fully processed antigen and is recognized by T-cells as such; (2) it is known to be one of the most powerful hapten "carriers" in the linked recognition mode; and (3) it can be used in humans without further testing.

As hapten-carrier binding agents, those conventionally employed in the preparation of antigens can be employed. The covalent coupling of the mimobodies of the invention to the immunogenic carrier material can be carried out in conventional manner. For example, for direct covalent coupling it is possible to utilize bis-N-succinimidyl derivatives, most preferably bis(sulfosuccinimidyl)suberate as coupling agent, or glutaraldehyde or carbodiimide, most preferably (dicyclohexyl)carbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

The ratios of hapten, hapten-carrier binding agent and carrier can be appropriately determined but it is preferred that the carrier be in an amount of about 1 to about 6 times, preferably about 1 to about 5 times the weight of the hapten, and the hapten-carrier binding agent be in an amount of about 5 to about 10 times the molar equivalent of the hapten. By the above coupling reaction, the carrier is bound to the hapten via the hapten-carrier binding agent to obtain a desired antigen composed of a peptide-carrier complex of mimotope of the invention and carrier.

After completion of the reaction, the resultant immunogen can be isolated and purified in conventional manner, such as by dialysis, gel filtration or fractionation precipitation.

The present invention is essentially directed to active immunization by direct vaccination; however, it also contemplates passive immunization. In such situation, mimobodies of the invention are administered to a suitable non-human animal and antibodies generated thereagainst are isolated and purified, and subsequently administered to a human subject for inducing alleviation of allergic symptoms.

The mimobodies of the invention are indicated for use as pharmaceuticals, especially vaccines, in particular in the treatment of IgE-mediated diseases, such as allergy, e.g. asthma, atopic dermatitis, allergic forms of eosinophilia, rhinitis, chronic urticaria and food allergies.

"Treatment" is to be understood as comprising prophylactic as well as curative treatment. The host is preferably human, but the invention is applicable mutatis mutandis to essentially any mammal, e.g. a cat or a dog.

Explanation of the Figures:

Figure 1: Interaction between IgE and its high affinity receptor.

Figure 2: Properties of the monoclonal anti-hIgE antibody BSW17.

Figure 3: The anti-idiotypic network:

The hIgE epitope recognized by BSW 17 and the anti-idiotypic paratope are schematically indicated as black dots. Black circles indicate the homologous hypervariable regions of antibody BSW17 (Ab1) and polyclonal antibodies 3 (Ab3), induced by immunization with the anti-idiotypic antibody Ab2.

Figure 4: Structure of three recombinant BSW17 mimobodies:

A: Anti-id-BSW17, clone 52 (SDS426); light chain: $(L.C.)_2$ (Seq.id. no. 36)
B: Anti-id-BSW17, clone 52 (SDS427); Fab: F_{AB} (Seq.id. no. 35 and 36)
C: Anti-id-BSW17, clone 43 (SDS463); Fab: F_{AB} (Seq.id. no. 37 and 38)

Figure 5: Anti-BSW17 Fab clones: DNA sequence of bacteriophage-displayed human immunoglobulin and deduced amino acid sequence:

Hypervariable regions (Complementarity Determining Regions; CDR) are in italics:
Figure 5a: clone 52; variable heavy chain (Seq.id.no. 1and 2) (CDR1: Seq.id.no. 39 and 40; CDR2: Seq.id.no. 41 and 42; CDR3: Seq.id. no. 43 and 44);
Figure 5b: clone 52; variable light chain (Seq.id.no. 3 and 4) (CDR1: Seq.id.no. 45 and 46; CDR2: Seq.id.no. 47 and 48; CDR3: Seq.id.no. 49 and 50);
Figure 5c: clone 43; variable heavy chain (Seq.id.no. 5 and 6) (CDR1: Seq.id.no. 51 and 52; CDR2: Seq.id.no. 53 and 54; CDR3: Seq.id.no. 55 and 56);
Figure 5d: clone 43; variable light chain (Seq.id.no. 7 and 8) (CDR1: Seq.id.no. 57 and 58; CDR2: Seq.id.no. 59 and 60; CDR3: Seq.id.no. 61 and 62).

Figure 6: Amino acid sequence homology between anti-BSW17 rFab and the Cε3 domain of human IgE:

A: anti-id Fab, clone 52, heavy chain (hIGE,Cε3: Seq.id.no. 25; clone 52: Seq.id.no.26);
anti-id Fab, clone 52, light chain, alignment 1 (hIGE,Cε3: Seq.id.no. 27;
clone 52: Seq.id.no.28);
anti-id Fab, clone 52, light chain, alignment 2 (hIGE,Cε3: Seq.id.no. 29;
clone 52: Seq.id.no.30), respectively.;

B: anti-id Fab, clone 43, heavy chain (hIGE,Cε3: Seq.id.no. 31; clone 43: Seq.id.no.32);
anti-id Fab, clone 43, light chain (hIGE,Cε3: Seq.id.no. 33; clone 43: Seq.id.no.34).
respectively.

Amino acid sequence alignment: identical residues are shown in black boxes, similar amino acids are in gray boxes (Lipman and Pearson). The positions of Fcε residues are indicated on top of each alignment. The contribution of hypervariable (CDR) and framework regions (FR) of the recombinant Fab fragments are shown below each pair of sequences.

Figure 7: Competitive binding of anti-id BSW17 rFab on IgE-primed CHOα cells with FITC-labeled BSW17

Figure 8: Binding of affinity-purified rabbit anti-BSW17 mimobody immunoglobulins to human IgE:

Determination of hIgE / anti-mimobody complexes by sandwich-ELISA: hIgE and immunoaffinity-purified anti-BSW17 mimobody preparations were mixed at equimolar concentration and incubated overnight at 4°C. The incubation mixtures were subsequently added to microtiter plate wells coated with monoclonal anti-hIgE antibody LE27 (1 µg/ml) as capturing antibody. Bound mimobody IgG was detected with goat anti-rabbit IgG-HRP:

□ = hIgE / SDS410 complexes;
o = hIgE / SDS411 complexes.

Figure 9: Anti-BSW17 mimobody immune response in Balb/c mice:

■ = mouse 1; ● = mouse 2; ▼ = mouse 3; ▲ = mouse 4; ◆ = mouse 5
A: anti-id-BSW17.52; light chain (SDS426);
B: anti-id-BSW17.52; Fab (SDS427);
C: anti-id-BSW17.43; Fab (SDS463);

O.D. values represent the optical density readouts, corrected for background binding to non-coated wells. Mean values of measurements in duplicate are shown. Variations were generally < 0.05 O.D.

Figure 10: Inhibition of HIgE / FcεRIα binding by immunoaffinity-purified anti-mimobody antibodies:

A: ■ = BSW17
● = anti-clone 52; light chain (SDS410)
Δ = anti-clone 52; Fab (SDS411)
▼ = anti-clone 52; light chain (column flowthrough)
B: ■ = BSW17
● = anti-clone 43 Fab (SDS476)

Figure 11: PCA score profiles of rhesus monkey groups (n = 2), immunized with various mimobody preparations:

A: Immunogen: anti-id-BSW17.52; light chain (SDS426);
B: Immunogen: anti-id-BSW17.52; Fab (SDS427);
C: Immunogen: anti-id-BSW17.43; Fab (SDS463).

Passive cutaneous anaphylaxis (PCA) reaction in rhesus monkey skin at various time points after immunization. PCA score values represent PCA intensities calculated from the area under the curves (AUC) generated by plotting the diameters of the blue skin dots against the injected IgE (JW8) concentrations. Scores are average numbers for each group of two monkeys immunized with the same mimobody preparation, calculated from the single monkey values shown in **Table 4**. Variations are shown as error bars. Statistical p values are indicated above error bars. Time points of boosting injections are indicated below the x-axis.

Figure 12: Complete amino acid sequences for the heavy and light chain of the three recombinant BSW17-mimobodies:

Figure 12a: Anti-id-BSW17, clone 52: variable and first constant domains of heavy chain (Seq.id. no. 35);
Figure 12b: Anti-id-BSW17, clone 52: variable and constant domains of kappa light chain (Seq.id. no. 36);
Figure 12c: Anti-id-BSW17, clone 43: variable and first constant domains of heavy chain (Seq.id. no. 37);
Figure 12d: Anti-id-BSW17, clone 43: variable and constant domains of lambda light chain (Seq.id. no. 38).

The mimobody $(L.C.)_2$ clone 52 comprises the light chains of Figure 12b (Seq.id. no. 36) bound by disulfide bridges as indicated in **Figure 4A**.

The mimobody Fab clone 52 comprises the light chain of Figure 12b (Seq.id. no. 36) and the heavy chain of Figure 12a (Seq.id. no. 35), bound by disulfide bridges as indicated in **Figure 4B**.

The mimobody Fab clone 43 comprises the light chain of Figure 12d (Seq.id. no. 38) and the heavy chain of Figure 12c (Seq.id. no. 37), bound by disulfide bridges as indicated in **Figure 4C**.

The following Examples illustrate the invention and are not limitative.

Temperatures are in degrees Celsius. The following abbreviations are used:

anti-id	=	anti-idiotypic
ABTS	=	[2,2'-azinodi(3-ethyl-benzthiazoline) sulphonate]
BSA	=	bovine serum albumin
BSW17	=	mouse monoclonal anti-human IgE antibody; Cε3 specific
CDR	=	complementarity determining regions
Cε3	=	third heavy chain constant region domain of IgE
Cε4	=	fourth heavy chain constant region domain of IgE
CεE	=	mimotope peptide mimicking the Cε3 epitope region of BSW17
CεM	=	mimotope peptide mimicking the Cε4 epitope region of BSW17
cfu	=	colony-forming units
ELISA	=	enzyme linked immunosorbent assay
Fab	=	antibody fragment lacking heavy chain constant regions 2 and 3
FcεRI; IgERI	=	high affinity receptor for IgE
FcεRIα	=	high affinity receptor for IgE, α chain
FCS	=	fetal calf serum
FR	=	framework regions
FITC	=	fluoresceine isothiocyanate-conjugated
HRP	=	horse radish peroxidase
HSA	=	human serum albumin
(h)IgE	=	(human) immunoglobulin E
mAb	=	monoclonal antibody
MNC	=	mononuclear cells
NIP	=	3-nitro-4-hydroxy-iodophenyl acetic acid
p.c.	=	polyclonal
Phab	=	phage displaying Fab fragments (Fab expressing bacteriophage)
PBS	=	phosphate-buffered saline
PCA	=	passive cutaneous anaphylaxis
PWM	=	pokeweed mitogen
r	=	recombinant
RT	=	room temperature
SPR	=	surface plasmon resonance

Example 1: Construction of phage display libraries

a) Source of lymphocytes

Two male adult donors were used to prepare mononuclear cells (MNC) from peripheral blood. A first male adult atopic donor who had clinical symptoms of allergy was boosted with an intramuscular injection of 0.5 ml of alum-adsorbed tetanus toxoid (Te Anatoxal Bern, Swiss Serum and Vaccine Institute, Bern, Switzerland). The MNC were isolated 7 days later using Ficoll gradient centrifugation (Lymphoprep, Pharmacia, Milwaukee, WI, USA) and then cultured for 3 days in RPMI-1640 medium (Seromed, Basel, Switzerland) containing 10^3 U/ml of IL-2 (Sigma, St-Louis, MO, USA), 50 µg/ml Pansorbin cells (Staphylococcus aureus Cowan strain1, Calbiochem, La Jolla, CA, USA) and tetanus toxoid diluted at 1:1000 in RPMI-1640 medium. Total RNA was then prepared from these cells using a phenol-chloroform guanidium isothiocyanate procedure (Chomczynsi, P. and Sacci, N., Anal. Biochem. 162 [1987] 156). The second male adult donor, a hyperimmune Rhesus D donor, was given an i.v. boost of 2 ml of packed red blood cells from a known male donor of blood group 0 RhD+. The MNC were isolated by Ficoll gradient centrifugation at +18 days after the boost. The cells were first cultured for 3 days in RPMI-1640 medium containing 10^3 U/ml of IL-2 and 10 µg/ml of pokeweed mitogen (PWM; Sigma L9379, Buchs, Switzerland) before extracting RNA.

Human tonsil samples were obtained from three tonsillectomized children. Tonsils were macerated in RPMI-1640 medium in sterile petri dishes and cut into little pieces. Tissue, cells and medium were then transferred into sterile tubes, the tissue debris allowed to settle and MNC were isolated from the supernatant using Ficoll gradient centrifugation. B cells were selected by incubating the MNC with CD19-coated paramagnetic beads and then RNA was prepared according to the phenol-chloroform guanidium isothiocyanate procedure mentioned above.

The pComb3 vector used for cloning of the chains for all mimobodies was obtained from the Scripps Research Institute La Jolla, CA, USA (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2 [1991] 119). The Escherichia coli strain XL1-Blue used for transformation of the pComb3 vector and the VCSM13 helper phage were purchased from Stratacyte (La Jolla, CA, USA).

b) Construction of bacteriophage libraries

Three separate libraries were constructed: the first one called **BS** from the MNC isolated from the first male atopic donor, the second one called **LD2** from the MNC harvested at +18 days after i.v. boost from the second male donor and the third one called **CT** from the B-cell enriched population of MNC isolated from the children's tonsils. Total RNA was prepared from these cells using the phenol chloroform guanidium isothiocyanate method. From this RNA, 10 µg were used to make cDNA using an oligo(dT) primer (400 ng) and reverse transcribed with M-MuLV reverse transcriptase according to the conditions specified by the supplier (Boehringer Mannheim, Germany). PCR amplification was performed as described in Vogel, M. et al., E.J. of Immunol. 24 (1994) 1200. Briefly, 100 µl of PCR reaction medium contained Perkin-Elmer buffer with 10 mM $MgCl_2$, 5 µl cDNA, 150 ng of each appropriate 5' and 3' primer, all four dNTP at 200 µM each and 2 U/ml Taq Polymerase (Perkin Elmer, NJ, USA). The PCR amplification of the heavy and light chains of the Fab molecule was performed separately with a set of primers from Stratacyte (details given below). For the heavy chain six upstream primers were used that hybridize to each of the six families of the V_H genes, whereas one kappa and one lambda chain primer were used for the light chains. The downstream primers were designed to match the hinge region of the constant domains γ1 and γ3 for the heavy chain. For the light chain the downstream primers were matched to the 3' end of kappa and lambda constant domains. The heavy and light chain PCR products were pooled separately, gel-purified and cut with Xho1/Spe1 and Sac1/ Xba1 restriction enzymes (Boehringer Mannheim, Germany), respectively. After digestion the PCR products were extracted once with phenol : chloroform : isoamyl alcohol and purified by gel excision. The insertion of the Xho1/Spe1 digested Fd fragment and subsequent ligation of the Sac1/Xba1 digested light chain into the pComb3 vector, the transformation into XL1-Blue cells, and the production of phages were performed as described in Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2 [1991] 119. After transformation of the XL1-Blue E.coli cells samples were withdrawn and titrated on plates to determine the library size. These results indicated expression libraries of 1×10^7, 7.7×10^6 and 3×10^6 cfu (colony forming units) for BS, LD2 and CT respectively.

c) PCR Primers

VHI 5′-CAC TCC CAG GTG CAG CTG CTC GAG TCT GG-3′
(Seq.id.no. 9);

VHII 5′-GTC CTG TCC CAG GTC AAC TTA CTC GAG TCT GG-3′
(Seq.id.no. 10);

VHIII 5′-GTC CAG GTG GAG GTG CAG CTG CTC GAG TCT GG-3′
(Seq.id.no. 11);

VHIV 5′-GTC CTG TCC CAG GTG CAG CTG CTC GAG TCG GG-3′
(Seq.id.no. 12);

VHV 5′-GTC TGT GCC GAG GTG CAG CTG CTC GAG TCT GG-3′
(Seq.id.no. 13);

VHVI 5′-GTC CTG TCA CAG GTA CAG CTG CTC GAG TCA GG-3′
(Seq.id.no. 14);

CHI(γI) 5′-AGC ATC ACT AGT ACA AGA TTT GGG CTC-3′
(Seq.id.no. 15);

VL(κ) 5′- GT GCC AGA TGT GAG CTC GTG ATG ACC CAG TCT CCA-3′
(Seq.id.no. 16);

CL(κ) 5′- T CCT TCT AGA TTA CTA ACA CTC TCC CCT GTT GAA GCT
CTT TGT GAC GGG CGA ACT C-3′
(Seq.id.no. 17);

VL(λ) 5′- C TGC ACA GGG TCC TGG GCC GAG CTC GTG GTG ACT CA-3′
(Seq.id.no. 18);

CL(λ) 5′- G CAT TCT AGA CTA TTA TGA ACA TTC TGT AGG GGC-3′
(Seq.id.no. 19).

Example 2: Selection of recombinant BSW17-specific antibody fragments (BSW-17-mimobodies) from phage libraries

Selection of BSW17-specific phages was carried out by performing four rounds of panning. Each round comprised two pre-absorptions on the anti-IgE mAb Le27 before absorption on the anti-IgE mAb BSW17. Preabsorption was performed as follows: two immunotubes (Maxisorp, Nunc) were coated with 4 ml of Le27 (20 µg/ml) overnight at 4°, then blocked for 2 h at 37° with 4 ml of PBS/2% skimmed milk. A first tube was incubated on a under-and-over-turntable at RT for 30 min with 4 ml of blocking solution containing 2×10^{12} cfu of each phage library (BS, LD2 and CT). The phages were then transferred into the second tube and the process was repeated once. After the second pre-absorption the non-Le27-specific phages were added to a tube coated with 4 ml of BSW17 (20 µg/ml) and blocked with PBS/2% skimmed milk as described above. After incubation for 2 h at RT on a under-and-over-turntable the tube was washed successively 10 times with PBS/0.1% Tween and 10 times with PBS. The adherent phages were eluted successively with, first, 500 µl of 0.1 M triethylamine and then after three times rinse in PBS, with 500 µl of 0.1M HCl adjusted to pH 2.2 with glycine and containing 1 mg/ml BSA. Each elution step was carried out for 10 min at RT and the eluted phages were neutralized with 250 µl of 1M Tris.Cl, pH 7.4 and 30 µl of 2 M Tris base, respectively. The selected phages were amplified using E. coli XL1-Blue cells as described in Barbas and Lerner, supra (1991) before being subsequently used in three more rounds of panning. After each round of panning the titer of eluted phages was monitored by cfu determination (**Table 2**):

Table 2

Enrichment of BSW17 specific Phabs by consecutive rounds of panning

Round of panning[a)]	Number of eluted phages (cfu)
1	3×10^5
2	2×10^4
3	3×10^5
4	5×10^7

[a)] For each round of panning 6×10^{12} phage particles were preabsorbed twice in tubes coated with 20 µg/ml of Le27 followed by incubation in one tube coated with 20 µg/ml of BSW17

Example 3: Nucleotide sequence of recombinant BSW17-mimobodies

Plasmid DNA from selected phage clones was prepared using a Nucleotrap kit (Machery-Nagel, Düren, Germany) and nucleic acid sequencing was carried out on an ABI 373A sequencing system using a PRISM Ready Reactin DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Germany).

Primers used for sequencing of the heavy chain sequence were:

CHγ1 (5'-CGCTGTGCCCCCAGAGGT-3') (Seq.id.no. 20) and

pCH (5'-GGCCGCAAATTCTATTTCAAGG-3') (Seq.id.no. 21).

To obtain the light chain sequences the following primers were used:

Cλ (5'-GAGACACACCAGTGTGGC-3') (Seq.id.no. 22),

Cκ (5'-CACAACAGAGGCAGTTCC-3') (Seq.id.no. 23) and

pCL (5'-CTAAACTAGCTAGTCGCC-3') (Seq.id.no. 24).

The primers were synthesized by Microsynth (Balgach, Switzerland). From the DNA sequences of a selection of various phage clones, two different amino acid sequences for BSW17-specific, recombinant antibody heavy and light chains were deduced (clone 52, clone 43). The sequences and their allocation to hypervariable regions (CDR) and framework sequences are shown in **Figures 5a** to **5d** (Seq.id.no. 1 to 8).

Alignment of the amino acid sequences of the BSW17-specific recombinant antibody fragments displayed by clones 52 and 43 with human IgE reveals homologies with stretches of the human Cε3 domain which is involved in binding to the high affinity receptor (**Figure 6**) (Seq.id.no. 25 to 34). Thus, the paratopes displayed by the recombinant antibody fragments mimic defined structures present in human IgE ("mimobodies"). Antibodies generated in allergic patients by vaccination with these recombinant mimobodies will therefore recognize human IgE and prevent allergic reactions by inhibiting IgE/IgERI binding.

Example 4: Preparation and purification of recombinant BSW17-mimobodies

Soluble mimobodies derived from phage clones 43 and 52 (**Figure 5**) (Seq.id.no. 1 to 8) were generated. In order to produce soluble Fab fragments the sequence gIII encoding the pIII tail protein of the phage particle was removed and replaced by a hexahistidine tag to facilitate the purification of the Fab fragment by Ni^{2+}-chelate affinity chromatography.

Phagemid DNA was prepared using a Nucleotrap kit (Macherey-Nagel, Düren, Germany) and digested with SpeI and NheI. The 4.7 kb DNA fragment lacking the gIII portion was treated with alkaline phosphatase and purified by agarose gel electrophoresis. The linearized DNA was ligated with a six histidine encoding DNA fragment at the 5' and 3' ends of SpeI and NheI restriction sites, respectively. After ligation the DNA was transformed into E. coli XL1-Blue cells and individual clones producing soluble mimobodies were selected. One of these clones was chosen for large scale purification and was grown in 1 l SB (Super Broth) containing 50 μg/ml carbenicillin at 37° to an OD of 1.0 at 600 nm. Culture was then induced with 1mM isopropyl β-D-thiogalactopyranoside (IPTG) (Biofinex, Praroman, Switzerland) and grown for 4 h at 37°. Bacteria were pelleted at 6000 rpm for 20 min at 4°, resuspended in 30 ml sonication buffer (0.1 M NaPO4, 8 M urea, pH 8.0) and subsequently sonicated on ice. Insoluble components were then removed by centrifugation at 15'000 rpm for 30 min at 4° and the supernatant containing Fab was purified on a 1 ml of nickel-nitriloacetate column. The column was washed with sonication buffer to remove contaminants followed by two elution steps with sonication buffer at pH 5.1 and 4.9, respectively. Fractions were monitored at OD 280 nm and aliquots were analysed by SDS-PAGE (12% non reducing) to confirm mimobody purity and identity. Fractions containing mimobodies were then pooled, concentrated and dialysed against PBS.

The purity of the final mimobody preparations (as specified in **Figures 4** and **12**) (Seq.id. no. 35, 36, 37, 38) was evaluated by assaying a sample by SDS-PAGE. Protein bands were detected by Coomassie blue staining. The concentration was determined by comparing the Coomassie blue stained mimobody band with known amounts of a standard protein (BSA) and also by spectrophotometry. These mimobody preparations were subsequently used for competitive binding assay and for immunization of rabbits.

Example 5: Inhibition of BSW17-mediated displacement of receptor-bound IgE by recombinant BSW17-mimobodies

BSW17 recognizes and displaces IgE bound to its high affinity receptor. In order to determine that the anti-id BSW17 rFab fragments are able to inhibit this displacement reaction, a competitive binding assay using IgE attached to cell surface exposed IgERI was performed. A recombinant chinese hamster ovary cell line (CHO), stably transfected with DNA encoding the α-chain of human IgERI was used for the assay [CHOα cell line; Blank, U. et al., Eur.J.Biol.Chem. 266 (1991) 2639]. A series of test samples containing 5 x 10^4 CHOα cells were incubated in FACS buffer (PBS, 0.3% BSA, 0.02% NaN_3) with 48 ng of IgE B11 hybridoma (Zürcher, A.W. et al., Immunol.Lett. 46 (1995) 49-57] for 15 min at RT. After washing once with FACS buffer each sample was incubated for 15 min at RT with preformed complexes of fluoresceine isothiocyanate-conjugated BSW17 (BSW17-FITC) and increasing amounts of anti-id BSW17 rFabs. The complexes were prepared as follows: 50 µl of BSW17-FITC at a concentration of 1.3 nM were incubated with different amounts of anti-id-BSW17 rFabs (40 nM; 200 nM; 1 µM; 4 µM; and 40 µM) for 30 min at RT. A control sample containing only the CHOα cells was incubated for 15 min at RT with BSW17-FITC for determination of non-specific binding. The CHOα cells were washed once with FACS buffer and after addition of 100 µl of FACS buffer the cells were analysed in a FACSCalibur (Becton Dickinson) flow cytometer equipped with an Argon laser tuned to 488nm. Gates in the forward scatter/side scatter dot blot were set around monomeric cells and fluorescence was quantitated and expressed as mean channel fluorescence (mcf). The percentage of positive cells was calculated as the percentage of BSW17 binding to CHOα cells. As seen in **Figure 7**, the binding of BSW17 to CHOα cells decreased with increasing concentrations of anti-id-BSW17 Fab fragments, indicating that the two anti-id BSW17 Fab clones were able to inhibit the binding of BSW17 to IgE.

Example 6: Immunization of rabbits with recombinant BSW17 mimobodies

This Example shows that immunization with either anti-BSW17 rFab (consisting of heavy plus light chain of clone 52 as shown in **Figure 4B** together with **Figures 12a** and **12b**) (Seq.id.no. 35 and 36), or the recombinant mimobody consisting of only the light chain (**Figure 4A** together with **Figure 12b**) (Seq.id.no. 36) induces in rabbits a humoral immune response which crossreacts with human IgE. Two New Zealand white female rabbits were given a primary immunization subcutaneously with 300 µg/ml anti-BSW17 rFab or light chain fragment of clone 52, emulsified 1:1 in Freund's complete adjuvant and then boosted three times with the same amount of mimobody emulsified 1:1 in Freund's incomplete adjuvant every two weeks. Sera were collected at day 0 (pre-bleed) and animals were bled 7 days after the last injection.

Rabbit immune sera were purified by immunoaffinity chromatography using human IgE (SUS-11 IgE), chemically crosslinked to Sepharose 4B colums. By this procedure the anti-hIgE fraction can be isolated from total immunoglobulins allowing accurate characterization of the therapeutically relevant immune response with respect to antibody titers and affinity.

The immunoaffinity purification of anti-mimobody antibodies which crossreact with human IgE consisted of two steps. In the first step the IgG fraction was isolated from the rabbit antiserum by ammonium sulfate precipitation, in the second step the hIgE-specific anti-BSW17 mimobody antibodies were bound to human IgE (SUS-11 IgE), covalently coupled to CH-Sepharose 4B, followed by elution, dialysis and concentration.

Concentration-dependent complex formation of the immunoaffinity-purified immunoglobulins with human IgE in solution was confirmed by ELISA: SUS-11 IgE was incubated with equimolar amounts of anti-mimobody immunoglobulins overnight at 4°. The complexes formed in solution were added to microtiter plate wells which had been coated with the monoclonal anti-hIgE antibody LE27 as capturing antibody. Bound anti-mimobody IgG was detected with polyclonal anti-rabbit IgG-HRP. The results are shown in **Figure 8**.

Example 7: Immunization of mice with recombinant BSW17 mimobodies

Recombinant mimobodies derived from both clone 43 and clone 52 can induce anti-mimobody antibodies. Immunizations were carried out in mice. Groups of five Balb/c mice were injected subcutaneously with 5 μg per mouse of recombinant BSW17 mimobodies which had been produced in E. coli bacteria and purified by nickel affinity chromatography. Aluminum hydroxide was used as adjuvant:

Group 1 was immunized with batch SDS426 = anti-Id-BSW17.52; light chain

Group 2 was immunized with batch SDS427 = anti-id-BSW17.52; Fab

Group 3 was immunized with batch SDS463 = anti-Id-BSW17.43; Fab

On days 21 and 41 after primary immunization, two booster injections (5 μg per mouse) were administered. Blood samples were taken on days 0, 20, 28, 35, 42, 49 and 56 after primary immunization. Serum was prepared and probed for the presence of anti-mimobody antibodies by ELISA.

Microtiter plate wells were coated with 1 μg / ml polyclonal human IgG and incubated with 1:50 diluted mouse sera prepared from blood samples taken at the indicated time points after primary immunization. Bound anti-mimobody antibodies (ahIgG, directed against the human framework and constant domain regions) were detected by a second incubation with horse radish peroxidase conjugated goat anti-mouse IgG (gamIgG-HRP). The colour reaction was developed using chromogenic ABTS substrate.

All mice produced anti-mimobody antibodies after the second boost with all recombinant mimobody preparations (**Figure 9**).

Example 8: Immunoglobulins generated in rabbits against recombinant BSW17 mimobodies bind to human IgE with high affinity

Vaccination with recombinant BSW17 mimobodies is shown here to induce a humoral immune response with high affinity for human IgE. The kinetic parameters representative for the binding of the immunoaffinity-purified anti-mimobody immunoglobulins to human IgE were analyzed by surface plasmon resonance (SPR).

SPR measurements were performed in a BIAcore instrument (Biacore, Uppsala, Sweden). Specific binding surfaces were prepared by coupling human IgE or murine IgG_3 to a CM5 sensor chip using amine coupling according to the manufacturer's instructions. Using this procedure, biomolecules are attached via primary amino groups to the carboxymethylated dextran surface of the sensor chip. 10 pmoles of human myeloma IgE or SUS-11 IgE were coupled to separate flow cells of the chip. The murine IgG_3 mAb, ABL 364 (ATCC HB 9324), was immobilized to a separate track of the same sensor chip. ABL 364 was used as a reference for determining binding of the anti-mimobody antibodies to non-hIgE immunoglobulin structures and to correct for possible changes of the refractive index caused by buffer changes. Coupling densities were ~ 13000 RU.

All biomolecular interactions measured in the BIAcore instrument were carried out at 25°, using HBS (10mM Hepes, pH = 7.4, 150 mM NaCl, 3.4 mM EDTA, and 0.005 % v/v surfactant P-20) as the continuous flow buffer. The concentration range of each analyte passed over the sensor chip surface for kinetic analysis was from 33 nM to 499 nM. The flow rate was 5μl/min. The analytes were injected for 1200 s, followed by HBS for approximately 1800 s to monitor the dissociation of bound analyte. The chip was regenerated with a 120 s pulse of 10 mM HCl. Non-specific binding was monitored by passing the analytes over the ABL 364 control track to be subtracted from specific binding prior to kinetic analysis.

Binding curves generated by SPR measurements were analyzed using the BIAevaluation 3.0 analysis package. For relative comparison of the anti-mimobody / IgE interactions a monophasic model was used. Association rate konstants k_a, dissociation rate constants k_d and the equilibrium dissociation constants $K_D = 1/K_A$ (K_A = affinity constant) were calculated for every curve and are summarized in **Table 3**:

Table 3

Kinetic constants (SPR / BIAcore) of the interaction between polyclonal (p.c.) rabbit anti-mimobody Ig preparations and hIgE

		Myeloma IgE	SUS-11 IgE
BSW17	K_a (1/Ms)	n.d.	$0.8 \pm 0.3 \times 10^4$
	K_d (1/s)	n.d.	$4.2 \pm 0.9 \times 10^{-6}$
	K_D(nM)	n.d.	0.53 ± 0.33
a(anti-id-BSW17.52;light chain)[1] (SDS410)	K_a (1/Ms)	$2.1 \pm 0.8 \times 10^4$	$2.2 \pm 0.9 \times 10^4$
	K_d (1/s)	$2.8 \pm 0.5 \times 10^{-4}$	$2.3 \pm 0.2 \times 10^{-4}$
	K_D (nM)	**13.3 ± 6.8**	**10.7 ± 3.3**
a(anti-id-BSW17.52;Fab)[2] (SDS 411)	K_a (1/Ms)	$4.6 \pm 2.7 \times 10^4$	$2.5 \pm 1.0 \times 10^4$
	K_d (1/s)	$2.3 \pm 0.3 \times 10^{-4}$	$1.5 \pm 0.1 \times 10^{-4}$
	K_D (nM)	**5.0 ± 2.2**	**6.0 ± 1.8**
a(anti-id-BSW17.43;Fab)[3] (SDS476)	K_a (1/Ms)	u.d.	$2.2 \pm 0.4 \times 10^4$
	K_d (1/s)	u.d.	$1.6 \pm 0.1 \times 10^{-5}$
	K_D (nM)	**u.d.**	**0.75 ± 0.02**

a = anti-
n.d. = not determined

[1] i.e. anti-SDS426
[2] i.e. anti-SDS427
[3] i.e. anti-SDS463

It appears that high affinity antibodies against human IgE could be induced in rabbits immunized with recombinant BSW17-mimobodies (K_D values in nanomolar range). Clone 43-derived Fab (SDS463; **Figure 4C**; anti-id-BSW17.43) has the capacity to induce an immune response of very high affinity for human IgE ($K_D < 1$ nM).

Thus by using a monoclonal anti-idiotypic antibody it was possible to induce a strong polyclonal response against IgE as intended by the human vaccination strategy.

Example 9: Immunoglobulins generated in rabbits against recombinant BSW17-mimobodies inhibit binding of human IgE to its high affinity receptor

For being active as an anti-allergy vaccine, complex formation between anti-mimobody antibodies and hIgE is expected to prevent IgE from binding to its high affinity receptor. The Cε3 epitope region Val(370) - Gly(379) in stretch Val(370-Asn(383) (**Figure 6**) (Seq.id.no. 25) which shows amino acid sequence homology with BSW17-mimobody CDR is involved in high affinity receptor binding. Therefore, IgE - specific antibodies raised against the recombinant BSW17 mimobodies are expected to exert their therapeutic effect by inhibiting binding of IgE to its high affinity receptor by blocking the binding domain. To confirm this, the purified anti-mimobody antibodies obtained from immunized rabbits were tested for inhibition of hIgE / FcεRIα binding in a competition ELISA. As a disease-relevant readout, free IgE was measured by its ability to bind to recombinant FcεRIα (RIα-HSA-RIα double fusion protein; DFP) (**Figure 10**).

hIgE (SUS-11; 1 μg/ml in Fig. 10A and 0.1 μg/ml in Fig. 10B) was pre-incubated 16 hrs at +4° with increasing amounts of anti-mimobody immunoglobulins or mAb BSW17 as a reference. The bulk immunoglobulins present in the immunoaffinity column flowthrough of the SDS410 preparation was included as a negative control. The formed complexes were added to microtiter plate wells coated with 1μg/ml of the anti-IgE antibody Le27 as a catching antibody and incubated with horse radish peroxidase labeled FcεRIα - HSA - FcεRIα double fusion protein (DFP) for 1 hr at 37°. Bound DFP was detected with a chromogenic substrate. O.D. values are expressed as % binding. Binding to competitor-free SUS-11 IgE was set as 100 %. Mean values of measurements made in duplicate are shown. Variations were generally below 2 %.

The results show that immunization with BSW17 mimobodies results in the generation of specific high affinity anti-hIgE antibodies in rodents. These anti-mimobody antibodies inhibit the binding of IgE to its high affinity receptor in vitro, indicating the value of the BSW17-mimobody vaccination strategy for anti-allergy vaccine development.

Example 10: Inhibition of passive cutaneous anaphylaxis by vaccination of rhesus monkeys with recombinant BSW17 mimobodies

Inhibition of passive cutaneous anaphylaxis (PCA) in monkeys can be used to test the anti-allergic activity of compounds in vivo.

Mimobody vaccination results in inhibition of anaphylactic skin reactions in rhesus monkeys. Groups of two monkeys were injected subcutaneously with 500 μg per animal of recombinant anti-BSW17 mimobodies. After primary immunization, two booster injections were administered. About 10 days after each boosting, PCA tests were performed. Monkeys VI 91, VI 92, VI 93 and VI 95 were tested for PCA reaction once more three months after the last boosting injection. The immunization scheme is summarized in the following **Table 1**:

Table 1

Monkey	Immunogen	Boosting (day no.)	PCA (day no.)
VI 91	anti-id-BSW17.52; light chain (SDS426) (Figure 4A)	0, 21, 54, 92	0, 30, 61, 105, 203
VI 92	anti-id-BSW17.52; light chain (SDS426) (Figure 4A)	0, 21, 54, 92	0, 30, 61, 105, 203
VI 93	anti-id-BSW17.52; Fab (SDS427) (Figure 4B)	0, 21, 54, 92	0, 30, 61, 105, 203
VI 95	anti-id-BSW17.52; Fab (SDS427) (Figure 4B)	0, 21, 54, 92	0, 30, 61, 105, 203
VI 2	anti-id-BSW17.43; Fab (SDS463) (Figure 4C)	0, 21, 54, 112	0, 33, 64, 123
VI 75	anti-id-BSW17.43; Fab (SDS463) (Figure 4C)	0, 21, 54, 112	0, 33, 64, 123

Rhesus monkeys pretreated with small doses of ketamine hydrochloride (10-15 mg/kg, i.m.) (Ketalar®, Parke Davis, GB) to keep them immobilized, received i.c. injections of various doses of IgE (JW8) (Serotec, Oxford, U.K.) into the skin of the abdomen. IgE (JW8) is a chimaeric antibody consisting of a mouse antigen-binding part specific for the hapten NIP and a human Fcε heavy chain. Increasing amounts (0, 2, 10, 50, 250 ng/ml saline) of IgE (JW8) were injected in a cephalocaudal series with a 30 gauge needle in a volume of 100 μl. Two hours later, 25 mg of NIP conjugated to BSA were administered

i.v. per animal. The animals were sedated again after the intravenous challenge with NIP-BSA conjugate. For the visualization of the skin reaction, Evans blue dye (1%, 0.5 ml/kg) was injected intravenously im mediately after the antigen challenge. The skin reactions were read 20 min after antigen injection by measuring two diameters of the blue spot and calculating their mean in mm.

PCA was tested at the indicated time points after primary immunization and boosting. PCA intensity was calculated from the area under the curves (AUC) generated by plotting the diameters of the blue skin areas against the injected IgE (JW8) concentrations. The PCA scores shown in **Table 4** for each single monkey represent the calculated AUC values:

Table 4

Effect of BSW17-mimobody vaccination on passive cutaneous anaphylaxis in rhesus monkeys

A

	PCA score			
Days after immunization	Immunogen: anti-id-BSW17.52; light chain (SDS426) (Figure 4A)		Immunogen: anti-id-BSW17.52; Fab (SDS427) (Figure 4B)	
	Monkey VI91	Monkey VI92	Monkey VI93	Monkey VI95
0	11.6 [1] (100) [2]	10.6 (100)	15.8 (100)	15.6 (100)
30	10.5 (91)	10.5 (99)	15.4 (97)	16.8 (108)
61	10.1 (87)	1.8 (17)	21.4 (139)	16.5 (106)
105	4.6 (40)	2.1 (20)	5.6 (35)	7.0 (45)
203	9.8 (84)	6.3 (59)	12.6 (80)	11.2 (72)

B

	PCA score	
Days after immunization	Immunogen: anti-id-BSW17.43; Fab (SDS463) (Figure 4C)	
	Monkey VI2	Monkey VI75
0	15.8 [1] (100) [2]	15.8 (100)
33	16.5 (104)	18.9 (120)
64	9.8 (62)	13.0 (82)
124	16.8 (106)	18.9 (120)

[1] PCA scores calculated from the area under the curves (AUC) generated by plotting the diameters of the blue skin areas against the injected IgE (JW8) concentrations as previously described (77)

[2] PCA intensity relative to pre-immunization values. PCA at day 0 = 100%

Taking the individual pre-immunization PCA scores (day 0 values) as a reference, all monkeys responded to BSW17 mimobody vaccination with a reduction of PCA intensity. Best results were obtained with the clone 52 light chain construct (SDS426) (Figure 4A) with inhibition rates of 60 – 83 % (PCA scores 40 and 17). Monkeys immunized with clone 52 Fab (batch SDS427) (Figure 4B) suppressed PCA by 55 – 65 % (PCA scores 45 and 35). A somewhat lower PCA inhibition ranging from 18 – 38 % (PCA scores 82 and 62) was observed with clone 43 Fab (batch SDS463) (Figure 4C), despite its superiority over clone 52 mimobodies with respect to BSW17 binding and high affinity anti-hIgE induction in rabbits.

Following on the vaccination protocol, vaccination with clone 52 mimobodies became effective after the third boosting injection (except monkey VI92) and partial PCA suppression was still observed more than 3 months later. In contrast, clone 43 Fab was effective after the second boosting injection, whereas a third mimobody challenge had no effect.

The average PCA score profile for each group of two monkeys immunized with the same mimobody preparation, calculated from the single monkey values of **Table 4**, is shown in **Figure 11**.

A positive PCA result in vaccinated monkeys (relative to individual pre-treatment scores or untreated control animals) is a clear indication for the efficacy of mimobody vaccination and proves the validity of its mechanistic concept. Vaccination of rhesus monkeys with recombinant BSW17 mimobodies results in the generation of high affinity anti-human IgE antibodies which inhibit PCA in vivo.

Claims:

1. An antibody or antibody fragment which is anti-idiotypic to an antibody that interferes with the binding of the Cε3 region of IgE to the high affinity receptor for IgE (a mimobody).

2. A mimobody according to claim 1 which is a recombinant, monoclonal antibody fragment anti-idiotypic to antibody BSW17.

3. A mimobody according to claim 1 consisting of or comprising the light chain dimer or one of the two Fab fragments of **Figure 4** (Seq.id. no. 35, 36, 37 and 38), whereby the constant regions therein also cover minor steric modifications such as found in allotypic variants.

4. A mimobody according to claim 1 consisting of or comprising an amino acid sequence of **Figure 5a**, **5b**, **5c** or **5d** (Seq.id.no. 2,4, 6 and 8).

5. A mimobody according to claim 1 consisting of or comprising at least one CDR, or preferably two, or more preferably the three CDR of **Figure 5a** (Seq.id.no. 2 and no. 40, 42 and 44), **5b** (Seq.id.no. 4 and no. 46, 48 and 50), **5c** (Seq.id.no. 6 and no. 52, 54 and 56) or **5d** (Seq.id.no. 8 and no. 58, 60 and 62), optionally together with adjacent framework sequences of up to about 10 amino acids at one or both CDR ends.

6. A mimobody according to claim 1 consisting of or comprising at least one of the sequences defined in **Figure 6** for clones 52 and 43 which are homologous to the Cε3 domain of human IgE (Seq.id.no. 26, 28, 30, 32 and 34).

7. A pharmaceutical composition such as a vaccine consisting of or comprising a mimobody according to any one of claims 1 to 6 either as a single molecular entity or as a protein conjugate chemically coupled to an immunogenic carrier molecule, where appropriate together with an adjuvant and further conventional excipients.

8. A mimobody according to any one of claims 1 to 6 for use as a pharmaceutical, in particular in the treatment of IgE-mediated diseases, such as allergy, in particular asthma, atopic dermatitis, rhinitis, chronic urticaria and food allergies, or the use of a mimobody according to any one of claims 1 to 6 in the preparation of a medicament against IgE-mediated diseases, in particular of a vaccine.

9. A method of treatment of IgE-mediated diseases by, in particular, vaccination, comprising administration of a therapeutically effective amount of a mimobody according to any one of claims 1 to 6 to a patient in need of such treatment or vaccination.

10. Use of a mimobody according to any one of claims 1 to 6 for raising polyclonal or monoclonal antibodies thereagainst by passive immunization; preparation of polyclonal or monoclonal antibodies against a mimobody according to any one of claims 1 to 6 for passive immunization, either by administration of a mimobody according to any one of claims 1 to 6 to a suitable non-human animal and isolation and purification of the antibodies generated thereagainst, or by conventional hybridoma technology; use of polyclonal or monoclonal antibodies whenever obtained from a mimobody according to any one of claims 1 to 6 in the treatment of IgE-mediated diseases by passive immunization; or use of an antibody such as BSW17 that interferes with the binding of the Cε3 region of IgE to the high affinity receptor for IgE, for the identification of a mimobody according to any one of claims 1 to 6.

Fig. 1

Interaction between IgE and its high affinity receptor

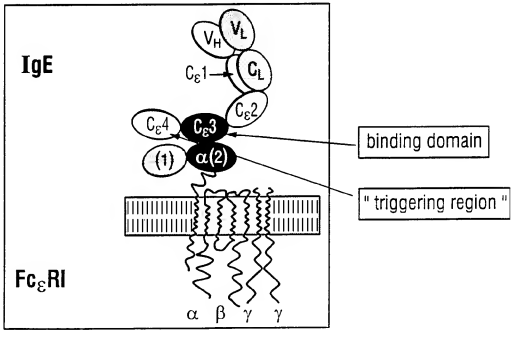

Fig. 2

Properties of the monoclonal anti-hIgE antibody BSW17

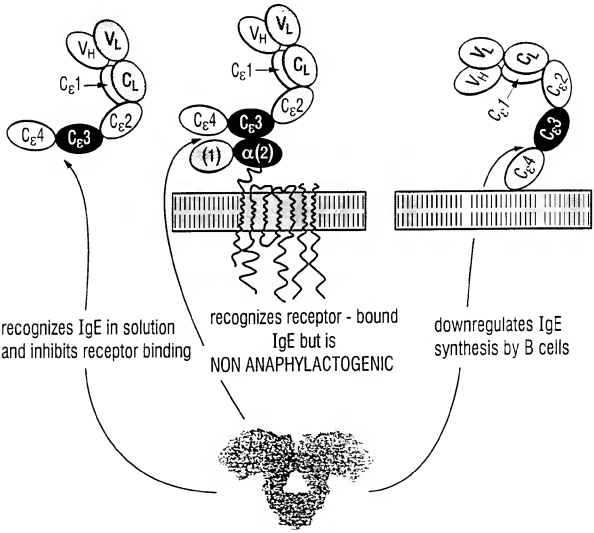

Fig: 3

The anti-idiotypic network

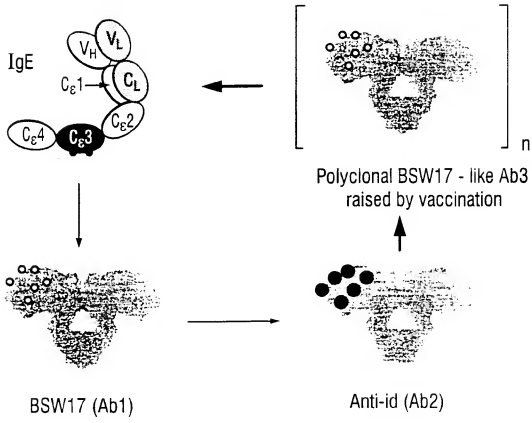

Fig. 4

Schematic structure of recombinant mimobodies

Fig.4A

Anti-id-BSW17.52; light chain

$(L.C.)_2$

Fig.4B

Anti-id-BSW17.52; Fab

F_{AB}

Fig.4C

Anti-id-BSW17.43; Fab

F_{AB}

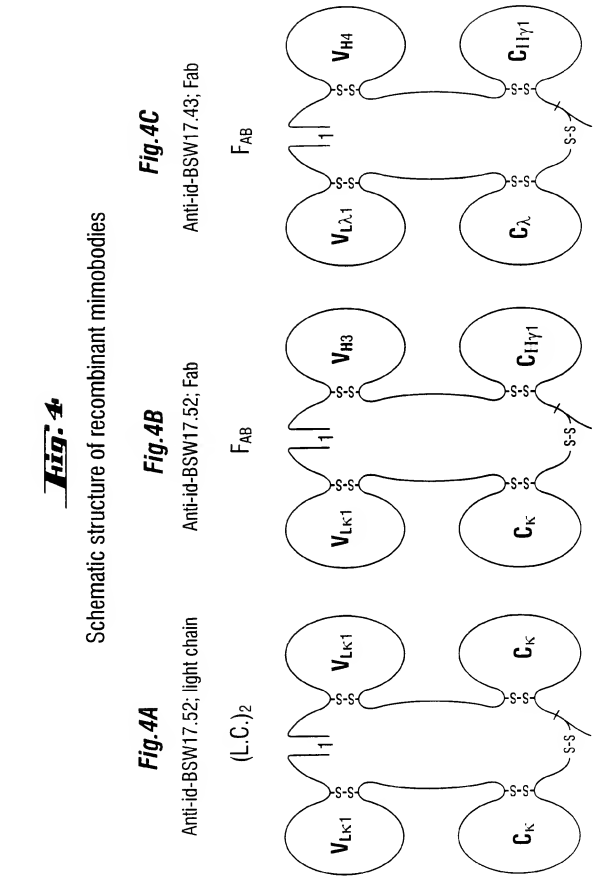

Fig. 5a

Anti-BSW17 Fab clone 52: variable heavy chain: DNA sequence of bacteriophage-displayed human immunoglobulin and deduced amino acid sequence. Hypervariable regions (Complementarity Determining Regions; CDR) are shown in italics.

			9			18			27			36			45			54
5′	CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	CTG	GTC	AAG	CCT	GGG	GGG	TCC	CTG
	Q	V	K	L	L	E	S	G	G	G	L	V	K	P	G	G	S	L
	AGA	CTC	63 TCC	TGT	GCA	72 GTC	TCT	GGA	81 TTC	ACC	TTC	90 AGT	CDR1 ***AAC***	***TAT***	99 ***AAT***	***ATG***	***AAC***	108 TGG
	R	L	S	C	A	V	S	G	F	T	F	S	***N***	***Y***	***N***	***M***	***N***	W
	GTC	CGC	117 CAG	GCT	CCA	126 GGG	AAG	GGA	135 CTA	GAG	TGG	144 GTC	TCA	CDR2 ***TCC***	153 ***ATT***	***AGT***	***AGT***	162 ***CGA***
	V	R	Q	A	P	G	K	G	L	E	W	V	S	***S***	***I***	***S***	***S***	***R***
	AAT	***TCT***	171 ***TAC***	***ATA***	***TAC***	180 ***TAC***	***GCA***	***GAC***	189 ***TCA***	***GTG***	***AAG***	198 ***GGC***	CGA	TTC	207 ACC	ATC	TCC	216 AGA
	N	***S***	***Y***	***I***	***Y***	***Y***	***A***	***D***	***S***	***V***	***K***	***G***	R	F	T	I	S	R
	GAC	AAC	225 GCC	GAG	AGT	234 ACC	TTG	TAT	243 CTG	CAA	ATG	252 GAC	AAC	CTG	261 GGA	GTC	GAA	270 GAC
	D	N	A	E	S	T	L	Y	L	Q	M	D	N	L	G	V	E	D
	ACG	GCT	279 GTC	TAT	TTT	288 TGT	ACG	AGC	CDR3 ***GGC***	***CGC***	***CTT***	306 ***TTC***	***GAC***	***TAC***	315 TGG	GGC	CAG	324 GGA
	T	A	V	Y	F	C	T	S	***G***	***R***	***L***	***F***	***D***	***Y***	W	G	Q	G
	ACC	CTG	333 GTC	ACC	GTC	342 TCC	TCT	3′										
	T	L	V	T	V	S	S											

Fig. 5b

Anti-BSW17 Fab clone 52: variable light chain: DNA sequence of bacteriophage-displayed human immunoglobulin and deduced amino acid sequence. Hypervariable regions (Complementarity Determining Regions; CDR) are shown in italics.

5′	GTG	ATG	9	CAG	TCT	18	TCC	TCA	27	TCT	GCA	36	GTA	GGA	45	AGA	GTC	54
	V	M	ACC T	Q	S	CCA P	S	S	CTG L	S	A	TCT S	V	G	GAC D	R	V	ACC T
	ATC	ACT	63	**CDR1**		72			81			90			99			108
	I	T	TGT C	***CGG*** ***R***	***GCT*** ***A***	***AGT*** ***S***	***CAG*** ***Q***	***GGC*** ***G***	***ATT*** ***I***	***AAC*** ***N***	***AAT*** ***N***	***TAT*** ***Y***	***TTA*** ***L***	***GCC*** ***A***	TGG W	TTT F	CAG Q	CAG Q
	AAA	CCA	117			126			135			**CDR2**			153			162
	K	P	GGG G	AAA K	GCC A	CCT P	AAG K	TCC S	CTG L	ATC I	TAT Y	***AGT*** ***S***	***GCA*** ***A***	***TCC*** ***S***	***ATT*** ***I***	***TTG*** ***L***	***CAA*** ***Q***	***AGT*** ***S***
	GGG	GTC	171			180			189			198			207			216
	G	V	CCA P	TCC S	AAG K	TTC F	AGC S	GGC G	AGT S	GGA G	TCT S	GGG G	ACA T	GAT D	TTC F	ACT T	CTC L	ACC T
	ATC	AGC	225			234			243			252			**CDR3**			270
	I	S	AAC N	CTG L	CAG Q	CCT P	GAA E	GAT D	TTT F	GCA A	ACT T	TAT Y	TAC Y	TGC C	***CAA*** ***Q***	***CAA*** ***Q***	***TAT*** ***Y***	***AAT*** ***N***
	TAT	***TAT***	279	***CTC***	***ACT***	288			297			306			315			
	Y	***Y***	***CCG*** ***P***	***L***	***T***	TTC F	GGC G	GGA G	GGG G	ACC T	AAG K	GTG V	GAG E	ATC I	AAA K	3′		

Fig. 5c

Anti-BSW17 Fab clone 43: variable heavy chain: DNA sequence of bacteriophage-displayed human immunoglobulin and deduced amino acid sequence. Hypervariable regions (Complementarity Determining Regions; CDR) are shown in italics.

```
5'  CAG GTG AAA CTG CTC GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG
     Q   V   K   L   L   E   S   G   P   G   L   V   K   P   S   E   T   L
                 9                  18                  27                  36                  45                  54

    TCC CTC ACC TGC ACT GTC TCT GGT GGC TCC ATC AGC AGT GGT TAC TAC TGG
     S   L   T   C   T   V   S   G   G   S   I   S   S   G   Y   Y   W
                63                  72                  81                 CDR1                 99                 108

    ACC TGG ATC CGC CAG CGC CCA GGG AAG GGC CTG GAG TGG ATT GGA TAC ATC TAT
     T   W   I   R   Q   R   P   G   K   G   L   E   W   I   G   Y   I   Y
               117                 126                 135                 144                 CDR2                162

    TAC AGT GGG AGC ACC TCC TAC AAC CCG TCC CTC AAG AGT CGA GTC ACC ATG TCA
     Y   S   G   S   T   S   Y   N   P   S   L   K   S   R   V   T   M   S
               171                 180                 189                 198                 207                 216

    GTG GAC ACG TCT AAA CAG TTC TCC CTG AGG CTG ACC TCT GTG ACT GCC GCG
     V   D   T   S   K   Q   F   S   L   R   L   T   S   V   T   A   A
               225                 234                 243                 252                 261                 270

    GAC ACG GCC GTC TAT TGT GCG CGA CGG GGT GGC TAC TAC TAC GGT ATG GAC GTC
     D   T   A   V   Y   C   A   R   R   G   G   Y   Y   Y   G   M   D   V
               279                 288                 297                 306                 315                 324

    CCC TAT TAC TAC ATA GAC GTC TGG GGC ACA GGG ACC ACC GTC ACC GTC TCC TCA 3'
     P   Y   Y   Y   I   D   V   W   G   T   G   T   T   V   T   V   S   S
               333                 342                 351                 360                 369                 378
```

Fig. 5d

Anti-BSW17 Fab clone 43: variable light chain: DNA sequence of bacteriophage-displayed human immunoglobulin and deduced amino acid sequence. Hypervariable regions (Complementarity Determining Regions; CDR) are shown in italics.

```
5'  GAG CTC      9 GTG ACT      18 CCT GCC      27 GTG TCT      36 TCT CCT      45 GGA CAG TCG      54
    E   L   V   V   T   Q   P   A   S   V   S   S   P   G   Q   S   I

    ACC ATC      63 TCC TGC     CDR1 72 ACC AGA      81 GAC GTT      90 GGT TAT      99 AAC TAT GTC      108
    T   I   S   C   T   G   T   R   S   D   V   G   Y   N   Y   V   S

    TGG TAC     117 CAA CAC     126 GGC AAA     135 CCC AAA     144 CTC ATG     153 CDR2 GAT GTC      162
    W   Y   Q   Q   H   P   G   K   A   P   K   L   M   I   Y   D   V   S

    AAT CGG     171 CCC TCA     180 TCT AAT     189 TTC TCT     198 GGC TCC     207 AAG TCT GGC AAC      216
    N   R   P   S   S   V   S   N   R   F   S   G   S   K   S   G   N   T

    GCC TCC     225 CTG ACC     234 GGG CTC     243 GCT GAG     252 GAC GAG     261 GCT GAT TAT TAC      270
    A   S   L   T   I   S   G   L   Q   A   E   D   E   A   D   Y   Y   C

    CDR3 279 TAT ACA     288 AGC ACT     297 CTC GGG     306 GTG TTC GGC GGA     315 GGG ACC AAG      324
    AGC TCA  S   Y   T   S   S   T   L   G   V   F   G   G   G   G   T   K   L
    S   S

    ACC GTC     333 CTA GGT CAG      342 CCC 3'
    T   V   L   G   Q   P
```

Fig. 6A

Amino acid sequence homology between anti-BSW17 rFab and the Cε3 domain of human IgE

Anti-Id Fab, clone 52; **heavy chain**:

370							379							383		
V	N	L	T	W	S	R	-	A	S	G	K	P	V	N	hIgE, Cε3	
Y	N	M	N	W	V	R	Q	A	P	G	K	G	L	E	clone 52	

CDR 1 FR2

Anti-Id Fab, clone 52; **light chain**; alignment 1:

343															358		
P	S	P	F	D	L	F	I	R	K	S	P	T	I	T	C	hIgE, Cε3	
P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	clone 52	

FR1

Anti-Id Fab, clone 52; **light chain**; alignment 2:

370								379							
V	N	L	T	W	S	R	A	S	-	G	hIgE, Cε3				
V	T	I	T	-	C	R	A	S	Q	G	clone 52				

FR1 CDR 1

Fig: 6B

Amino acid sequence homology between anti-BSW17 rFab and the Cε3 domain of human IgE

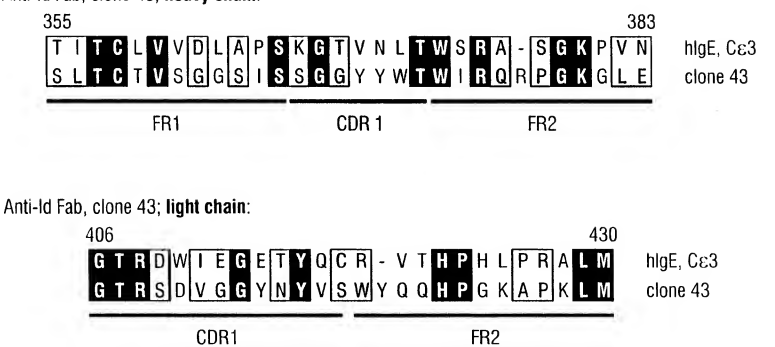

Fig. 7

Competitive binding of anti- id BSW17 rFab on IgE-primed CHOα cells with FITC-labeled BSW17

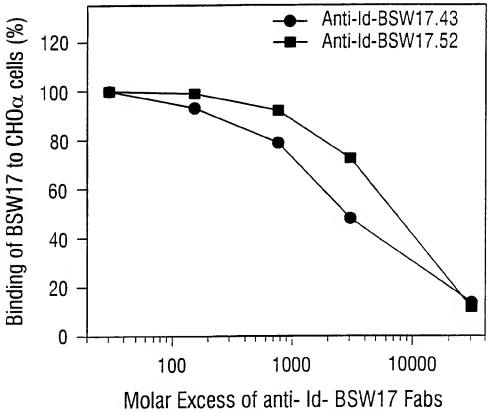

Fig. 8

Binding of affinity-purified rabbit anti-BSW17 mimobody immunoglobulins to human IgE

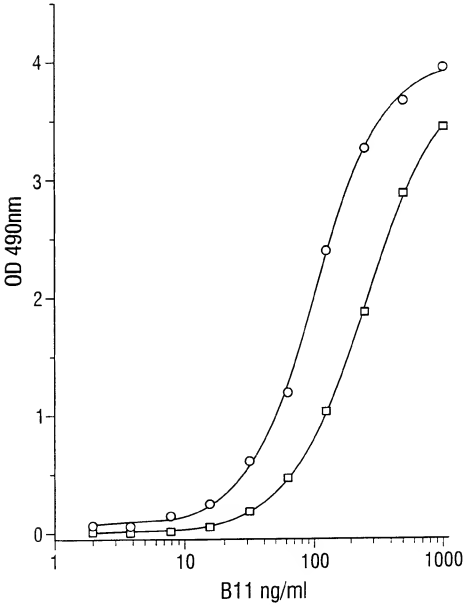

Fig. 9 Anti-BSW17 mimobody immune response in Balb/c mice

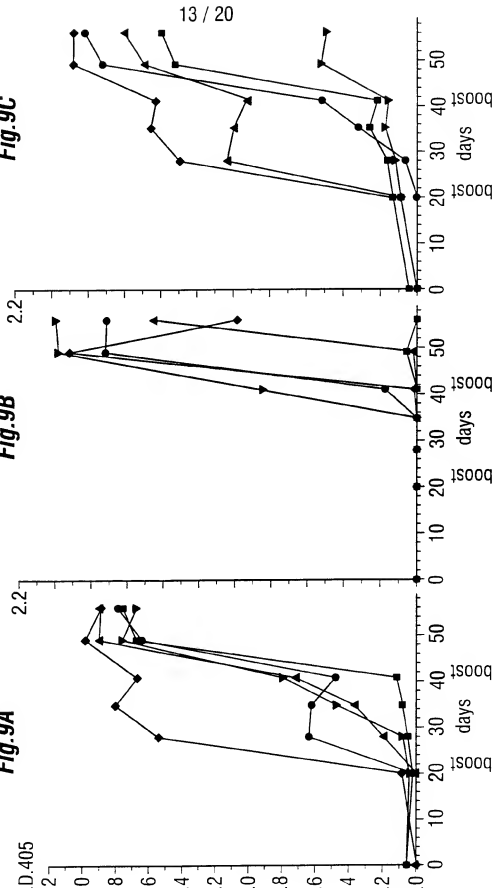

Fig. 10 Inhibition of hIgE / FcεRIα binding by immunoaffinity-purified anti-mimobody antibodies

Fig.10A

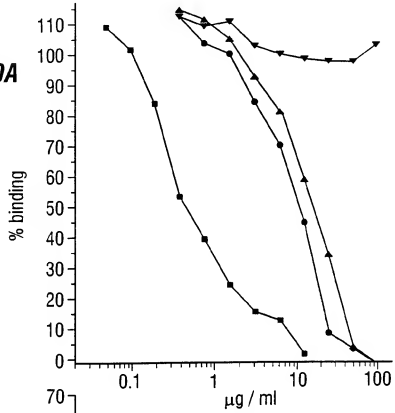

Fig.10B

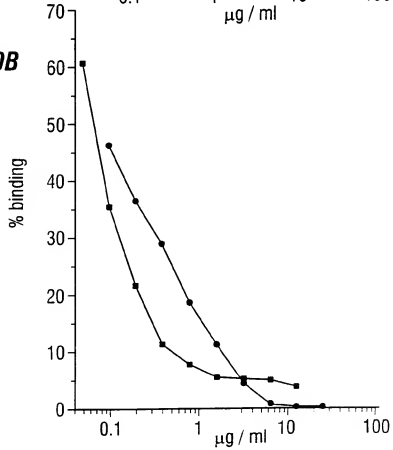

Fig. 11

PCA score profiles of rhesus monkey groups (n = 2), immunized with different mimobody preparations

Fig.11A Immunogen: anti-id-BSW 17.52; i.c. (SDS426) ***Fig.11B*** Immunogen: anti-id-BSW 17.52; Fab (SDS427)

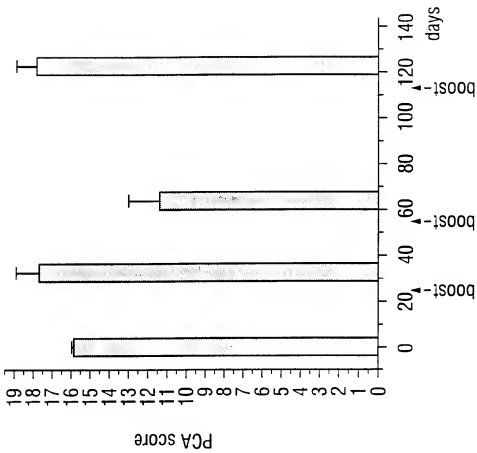

Fig. 11C Immunogen: anti-id-BSW 17.43; Fab (SDS463)

Fig. 12a

Anti-BSW17 Fab clone 52: variable and first constant domain of heavy chain

```
Q V K L L E S G G G L V K P G G S L R L S C A V S
G F T F S N Y N M N W V R Q A P G K G L E W V S S
I S S R N S Y I Y Y A D S V K G R F T I S R D N A
E S T L Y L Q M D N L G V E D T A V Y F C T S G R
L F D Y W G Q G T L V T V S S A S T K G P S V F P
L A P S S K S T S G G T A A L G C L V K D Y F P E
P V T V S W N S G A L T S G V H T F P A V L Q S S
G L Y S L S S V V T V P S S S L G T Q T Y I C N V
N H K P S N T K V D K K A E P K S C
```

Fig. 12b

Anti-BSW17 Fab clone 52: variable and constant domain of kappa light chain

V M T Q S P S S L S A S V G D R V T I T C R A S Q
G I N N Y L A W F Q Q K P G K A P K S L I Y S A S
I L Q S G V P S K F S G S G S G T D F T L T I S N
L Q P E D F A T Y Y C Q Q Y N Y Y P L T F G G G T
K V E I K T V A A P S V F I F P P S D E Q L K S G
T A S V V C L L N N F Y P R E A K V Q W K V D N A
L Q S G N S Q E S V T E Q D S K D S T Y S L S S T
L T L S K A D Y E K H K V Y A C E V T H Q G L S S
P V T K I F N R G E C

Fig. 12c

Anti-BSW17 Fab clone 43: variable and first constant domain of heavy chain

```
Q V K L L E S G P G L V K P S E T L S L T C T V S
G G S I S S G G Y Y T W W I R Q P P G K G L E W I
G Y I Y Y S G S T S Y N P S L K S R V T M S V D T
S K N Q F S L R L T S V T A A D T A V Y Y C A R E
R G E T G L Y Y P Y Y Y I D V W G T G T T V T V S
S A S T K G P S V F P L A P S S K S T S G G T A A
L G C L V K D Y F P E P V T V S W N S G A L T S G
V H T F P A V L Q S S G L Y S L S S V V T V P S S
S L G T Q T Y I C N V N H K P S N T K V D K K A E
P K S C
```

Fig. 12d

Anti-BSW17 Fab clone 43: variable and constant domain of lambda light chain

```
E L V V T Q P A S V S G S P G Q S I T I S C T G T
R S D V G G Y N Y V S W Y Q Q H P G K A P K L M I
Y D V S N R P S G V S N R F S G S K S G N T A S L
T I S G L Q A E D E A D Y Y C S S Y T S S S T L G
V F G G G T K L T V L G Q P Q P K A A P S V T L F
P P S S E E L Q A N K A T L V C L I S D F Y P G A
V T V A W K A D S S P V K A G V E T T T P S K Q S
N N K Y A A S S Y L S L T P E Q W K S H K S Y S C
Q V T H E G S T V E K T V A P T E C S
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Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Leu Gln Pro Glu Asp
65                  70                  75                  80
Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Tyr Tyr Pro Leu Thr Phe
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Gly Gly Gly Thr Lys Val Glu Ile Lys Thr Val Ala Ala Pro Ser Val
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Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
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INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 3, line 28 - 30

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
ECACC European Collection of Cell Cultures
Center for Applied Microbiology and Research

Address of depositary institution *(including postal code and country)*
Porton Down
Salisbury, SP4 0JG Wiltshire
United Kingdom

Date of deposit	Accession Number
December 19, 1996	96121916

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/EP 00/03288

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/42 C07K16/28 A61K39/395 A61P37/00 A61P17/00
A61P11/06 G01N33/577 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STADLER, BEDA M. ET AL: "Anti-IgE vaccination" PROG. ALLERGY CLIN. IMMUNOL., PROC. INT. CONGR. ALLERGOL. CLIN. IMMUNOL., 16TH (1997), 339-342. EDITOR(S): OEHLING, ALBERT K.;HUERTA LOPEZ, J. G. PUBLISHER: HOGREFE & HUBER, SEATTLE, WASH. , XP000916459 page 340, left-hand column, paragraph 3 -page 341, left-hand column, paragraph 2 --- -/--	1-10

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2000	08.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	von Ballmoos, P

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STADLER B M ET AL: "Mimotope and anti-idiotypic vaccines to induce an anti-IgE response." INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 JANUARY) 118 (2-4) 119-21. , XP000916451 abstract page 120 ---	1-10
X	VOGEL, M. ET AL: "Role of anti-idiotype antibodies in IgE regulation." TUMOR BIOLOGY, (SEPT., 1997) VOL. 18, NO. SUPPL. 2, PP. 59. MEETING INFO.: MEETING ON FROM BASIC CANCER RESEARCH TO CLINICAL APPLICATION HELD AT THE XXVTH ANNIVERSARY MEETING OF THE INTERNATIONAL SOCIETY FOR ONCODEVELOPMENTAL BIOLOGY AND MEDICINE MON, XP000916380 the whole document ---	1-10
X	WO 89 06138 A (TANOX BIOSYSTEMS INC) 13 July 1989 (1989-07-13) page 17, line 1 - line 11; claim 29 ---	1,7-10
X	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 04, 30 April 1999 (1999-04-30) & JP 11 000174 A (ASAHI BREWERIES LTD;TORII YAKUHIN KK; NIKKA UISUKII KK; RA TOMOYASU), 6 January 1999 (1999-01-06) abstract Sequence 1 on pages 5 and 6 -----	1,3

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/03288

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9 (complete) and 10 (partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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WO 00/63252

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir. ational Application No
PCT/EP 00/03288

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